Potential Modes of Action for Low-Dose Formaldehyde-Induced Cancer

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Abstract (Abbreviated)

Formaldehyde was recently classified by the International Agency for Research on Cancer as a human carcinogen based on epidemiological evidence for increased risk of nasopharyngeal carcinoma. A biologically motivated dose–response model based on animal studies suggests that saturation of alcohol dehydrogenase-3 (ADH3), cytotoxicity, and regenerative hyperplasia are proposed key events in a mode of action for development of respiratory tract tumors at the portal of entry. Epidemiological studies also indicate an increased risk for leukemia following formaldehyde inhalation exposure, which suggests that the currently proposed mode of action for nasal tumors may be inadequate for other cancers and perhaps incomplete with respect to nasal tumors. The primary enzymatic detoxification of formaldehyde involves spontaneous conjugation to glutathione, followed in turn by ADH3-catalyzed oxidation. More recently, however, it has been recognized that ADH3 also catalyzes the reduction of S-nitrosoglutathione, a molecule that mediates post-translational regulation of proteins via modification of cysteine residues. Evidences are presented that imply that low-dose effects of formaldehyde might include post-translational protein modifications and altered oxidative and nitrosative states. Such effects are known to influence growth, survival, and differentiation of cells; thus, these ideas may constitute a refinement to the hypothesized mode of action and provide insight into how low-level formaldehyde exposure might induce cancer without overt cytotoxicity or genotoxicity.

Evidence for Low Dose Proliferation

Animal bioassays indicate that regenerative hyperplasia is an important component to formaldehyde-induced carcinogenesis. Within such reports, however, *in vivo* evidence for formaldehyde-induced proliferation is observed in regions where epithelial lesions are not observable via light microscopy. *In vitro* evidence also supports that formaldehyde can increase cell proliferation, as low-dose formaldehyde exposure has been shown to increase cell growth and mitotic index and decrease apoptotic index; suggesting that this proliferation occurs in the absence of toxicity.

Oxidative Status

A potential explanation for this proliferation may involve mild oxidation, as it has been demonstrated in several cell types that the redox potential (i.e. the ratio of reduced and oxidized glutathione, GSH/GSSG) changes during phases of proliferation and differentiation. This ratio has been shown to effect post-translational *S*-glutathionylation of protein cysteine residues, and is one mechanism by which redox sensitive proteins like certain transcription factors sense their environment (**Figure A**). *In vitro*, some proteins appear to be *S*-glutathionylated, and exposure of cells to oxidants can significantly increase the number of *S*-glutathionylated proteins. Formaldehyde conjugates readily with GSH, and thus may directly affect the GSH/GSSG ratio in cells. **Figure B** depicts the GSH-dependent metabolism of formaldehyde.

Nitrosative Status

In addition to mediating formaldehyde clearance, ADH3 catalyzes the reduction of GSNO (**Figure B**). Like GSSG, GSNO modifies protein function via protein thiols (**Figure A**). This has been shown for metabolic enzymes, DNA repair enzymes, ion channels, and G-protein coupled receptors. Both *S*-glutathionylation and *S*-nitrosylation target protein cysteine residues, and recent evidence suggests that some proteins (e.g. calcium channels) are differentially modulated by each form of modification. We posit that formaldehyde-induced proliferation may involve perturbation of post-translational protein modification, as both formaldehyde and GSNO are substrates for ADH3.

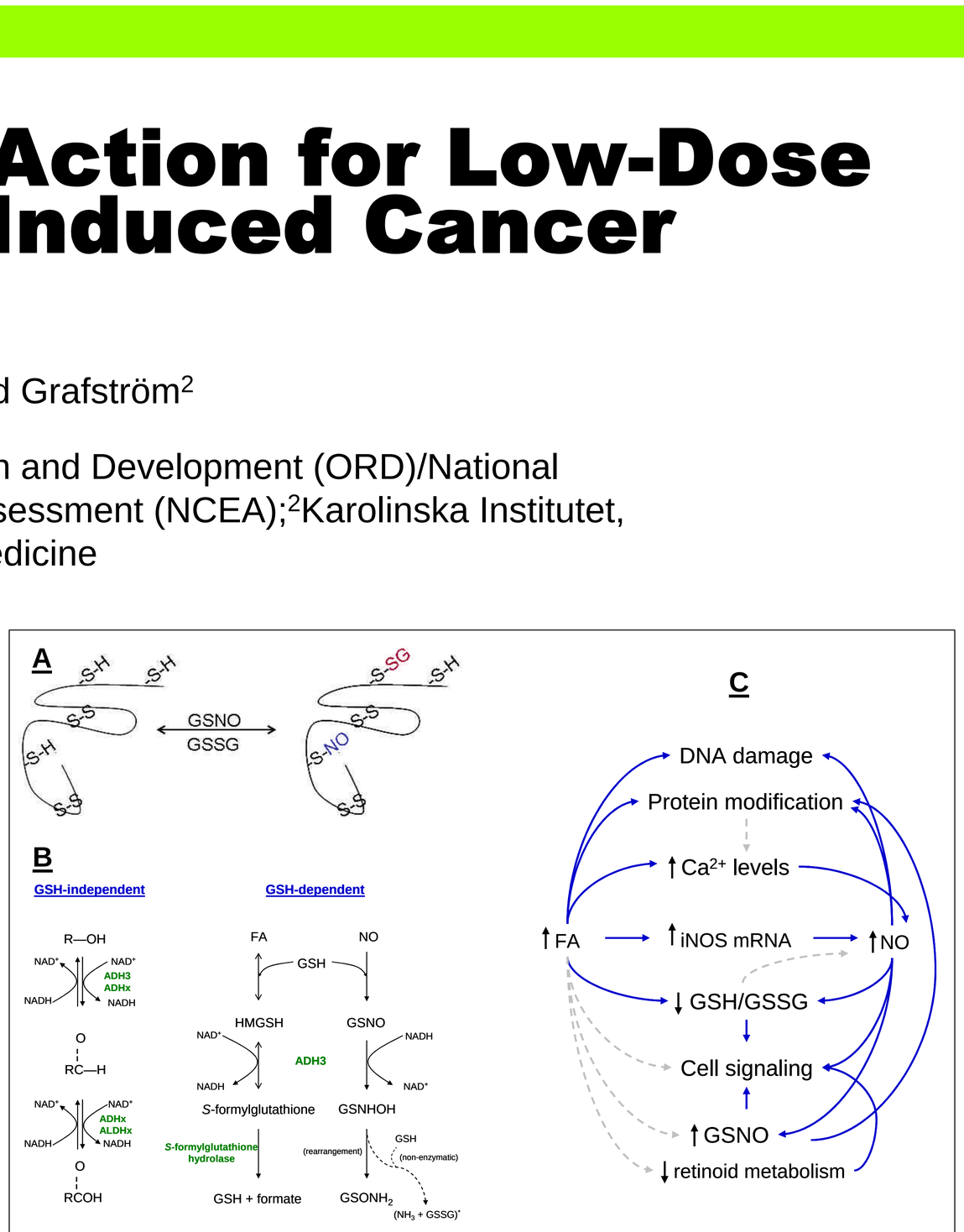

Figure. (A) Low molecular weight thiols (e.g. GSSG) and nitrosothiols (e.g. GSNO) can modify protein thiols by *S*-glutathionylation and *S*-nitrosylation, thereby forming protein mixed disulfides affecting protein structure/function. (B) Enzymatic activities of human ADH3. Note that this ubiquitous enzyme also metabolizes retinol. (C) Potential mechanisms by which formaldehyde may influence cell signaling: DNA damage, modify proteins, increase calcium levels, alter GSH pools and redox and nitrosative states. The dotted arrows represent present data gaps, and provide future lines of research to better characterize mechanisms of formaldehyde-induced cell growth, differentiation, and death. FA, formaldehyde; NO, nitric oxide; HMGSH, hydroxymethylglutathione; $GSONH_2$, glutathione sulfinamide; iNOS, inducible NO synthase.

Implications in Cell Signaling Mechanisms

Clearly, the role of ADH3-mediated reactions needs further consideration, including competition between formaldehyde and NO for free GSH, competition between substrates (e.g. HMGSH and GSNO) for ADH3, as well as from alteration of the NADH/NAD^+ redox couple that directs ADH3 function (**Figure B**). Also, ADH3 mRNA levels couple to proliferative status in cells, suggesting that proliferating cells need capacity to rapidly regulate one or more of these endogenous substrates. **Figure C** depicts some of the complex relationships (not all discussed herein) between formaldehyde and NO. Recognizing that both of these compounds can cause cytotoxicity at high doses, we posit that low-dose formaldehyde exposure may also cause changes (perhaps within the physiologic ranges) in oxidative or nitrosative states that result in noncytotoxic cell proliferation via cell signaling mechanisms, perhaps mediated by protein modification.

Conclusions

Saturation of ADH3, cytotoxicity, and regenerative hyperplasia are undoubtedly involved in high dose formaldehyde-induced carcinogenicity. Recent data on ADH3 metabolism, however, indicate that formaldehyde may influence several ADH3-mediated reactions with potential consequences on cell signaling. To our knowledge, toxicity studies to date have not investigated the potential impact of formaldehyde exposure on these related pathways. Important questions are whether this proliferation is transient or sustained, whether this component of proliferation is operational at both low and high dose exposures, and whether such proliferation contributes to cancer risk.

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